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The relationships of peruvian *Oxalis* species to cultivated oca

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Abstract

As part of an investigation of the origins of domestication and polyploidy in oca (***Oxalis tuberosa*** Molina) wild species of Oxalis were collected in the Andean highlands and cloud forest region of the eastern slopes in the Peruvian departments of Amazonas, Cajamarca, Ancash, Junín, Cusco and Puno. Cladistic analyses of data from DNA sequences of two genes (chloroplast-expressed glutamine synthetase "ncpGS" and the internal transcribed spacer of nuclear ribosomal DNA "ITS") of these species and others from Bolivia confirm the close relationship of a group of these wild species with cultivated oca. This group is in agreement with cytological data in the sense that the species of the x = 8 "***Oxalis tuberosa*** alliance" described by De Azkue and Martínez in 1990 are all grouped in a single clade in each of the cladograms for both genes. However, the group includes additional species for which there are as yet no published chromosome counts. The presence of multiple ncpGS sequences in individual plants of oca indicates that oca is an allopolyploid - a hybrid of several wild species. Some wild species with tubers share some of the same ncpGS sequences as those found in cultivated oca, so these are supported as possible progenitors of the cultigen.

The morphology of this alliance of ***Oxalis*** species related to oca is contrasted with other groups of more divergent species. In Peru, these groups of ***Oxalis*** less closely related to oca include the species of the sections ***Thamnoxys*** (plants with leaves with a developed rachis, most of them small shrubs growing at low elevations), ***Ionoxalis*** (acaulescent bulbous herbs) and ***Corniculatae*** (creeping weeds, some native and others cosmopolitan). Other species have intermediate relationships with oca and its relatives – for example, a group of species related to ***O. megalorrhiza*** Jacquin. These have perennial caudices (some with thickened roots, but not true tubers) and flowers with unequal sepals, and are found in dry habitats from sea-level to 4000 m.

The wild relatives of oca and of other crops are important genetic resources for the plant breeding of the future. The conservation of the species identified in these studies as relatives of oca is important since some are endangered by deforestation.

Introduction

The tuber crop "oca," ***Oxalis tuberosa*** Molina, is well known in the central Andean region, but is little known in most other areas of the world. Although undoubtedly of Andean

origin, its wild progenitors are still unknown. Because oca is an octoploid (e.g., de Azkue and Martínez, 1990; see below), the question of its origins includes not only the identification of the wild species that was domesticated, but also the study of the origins of polyploidy. Polyploids may be derived from a single species (autopolyploids) or from hybridization of more than one progenitor species or well-differentiated population (various kinds of allopolyploids). They may have arisen only once, but it is now known that most polyploids have originated in multiple events (Soltis and Soltis, 1993). The phylogenetic relationships of oca's close relatives can provide a framework for the exploration of these questions about the origins of domestication and polyploidy in ***O. tuberosa.***

The study of the wild relatives of crops can shed light on the processes of plant domestication and crop evolution, and can also have practical applications because the wild relatives of crops are important genetic resources for plant breeding. Traits that have been introduced to crops from related wild species include increased yield, resistance to pests and diseases, and improved nutritional, culinary, and processing qualities (Tapia, 1993). The first part of this paper summarizes the findings of my dissertation research project concerning the origins of cultivated oca and its relationships with wild ***Oxalis*** species, particularly those of the central Andean region. The ***Oxalis*** species that are identified as close relatives of oca in this and other studies may have potential utility for future oca breeding efforts by Andean researchers, and so the conservation of these species is important. The second part of this paper briefly contrasts some morphological features of this group of ***Oxalis*** species with other species of ***Oxalis*** that are also common in Peru, but which are less closely related to ***O. tuberosa.***

Background: Taxonomy and cytology

The search for the origins of oca is a difficult taxonomic challenge, in part because of the large size and confused taxonomy of the genus. *Oxalis* comprises over 800 species, most of them in South America and southern Africa, with over 80 species listed for Peru alone (Macbride, 1943; Pool in Brako and Zarucchi, 1993). Overall, South American *Oxalis* species embrace by far the greatest range of morphological, ecological, and cytological characteristics. The genus was classified into 38 sections by Knuth (1930) in his monograph in Engler's ***Das Pflanzenreich***, the most recent treatment of the entire genus, but these sections are widely recognized as artificial, and so they do not provide a good guide to the close relatives of oca. Most of the subsequent work on South American ***Oxalis*** has been done by Alicia Lourteig, of the Muséum National d'Histoire Naturelle in Paris, but her treatment of the section that includes ***O. tuberosa*** has not yet been published. Species delimitations are problematic in many parts of ***Oxalis***, especially since some characters can only be determined from living material (Salter, 1944). The large number of conflicting determinations in herbaria and the lack of good keys to the species make identification of specimens is difficult. Phenotypic plasticity and evidence of hybridization further complicate determinations, so some of the species names used here must be considered tentative.

Cytological work by de Azkue and Martínez (1983; 1984; 1988; 1990) contributed to the discovery of more natural groupings of ***Oxalis*** species than the sections of Knuth. They

reported chromosome numbers of a group they call the "***Oxalis tuberosa*** alliance" (de Azkue and Martínez, 1990), including a dozen morphologically similar species that share a base chromosome number, $x = 8$, that is rare in ***Oxalis***. Oca was the only species they studied that bore tubers, and was octoploid with $2n = 8x = 64$ (de Azkue and Martínez, 1990). Although there are some conflicting chromosome counts reported for ***O. tuberosa*** (e.g., Talledo and Escobar, 1995; Guamán C., 1997; reviewed in Emshwiller, 1999), 64 chromosomes have also been found in well over 100 cultivated oca accessions by other cytologists (Medina H., 1994; Valladolid et al., 1994; Valladolid, 1996; Ishiki, 1997).

The "***Oxalis tuberosa*** alliance" appears to include other species in addition to those studied by de Azkue and Martínez (1990). The species included in that study were ***O. tuberosa*** Molina, ***O. herrerae*** Knuth, ***O. medicaginea*** H.B.K., ***O. mollissima*** (Rusby) Knuth, ***O. oblongiformis*** Knuth, ***O. peduncularis*** H.B.K, ***O. subintegra*** Knuth, ***O. tabaconasensis*** Knuth, *O.* sp. aff. ***villosula*** Knuth, ***O. lotoides*** H.B.K., ***O. spiralis*** R. & P. ex G. Don, and one unidentified ***Oxalis*** species. A few other species have been reported with $x = 8$: ***O. ptychoclada*** Diels (Favarger and Huynh, 1965; Huynh, 1965), ***O. melilotoides*** Zuccarini (Brücher, 1969), and ***O. nubigena*** Walpers (Diers, 1961). In addition, the inclusion of other species that as yet lack cytological data is suggested by both morphological similarities and molecular analyses (see below). As pointed out by de Azkue and Martínez (1990), the "***Oxalis tuberosa*** alliance" is incongruent with the sectional classification of Knuth (e.g., 1930; 1931; 1935; 1936), the members being found in several different sections. Most of these sections also include species with different base chromosome numbers. For example, ***Oxalis ortgiesii*** Regel, the type species of the section ***Ortgieseae*** Knuth, in which Knuth (1930) classified oca, has 14 chromosomes, or $x = 7$ (Heitz, 1927; Warburg, 1938; Marks, 1956).

Background: Molecular systematics of polyploids

In phylogenetic analyses, the methods of cladistics are used to infer the evolutionary changes that occurred to produce the differences among taxa, and thus arrive at one or more hypotheses about the phylogenetic relationships among them. Phylogenetic trees are often represented graphically by cladograms, branching diagrams that show which taxa are hypothesized to be more or less closely related (i.e., which taxa are thought to share a more recent common ancestor and which diverged longer ago).

The methods of molecular systematics can be particularly useful in studies of both polyploid origins and crop evolution (Doebley, 1992; Soltis et al., 1992). In the analysis of DNA sequence data, molecular systematists compare sequences of the same gene from several different species. In this case it is the changes among the different sequences that are inferred by cladistic methodology, and phylogenetic relationships are reconstructed among the gene sequences themselves—called the "gene tree." The question of whether or not this gene tree actually represents the relationships of the species themselves may be complicated by many factors, such as hybridization and gene duplication, among others (reviewed in Doyle, 1992). Thus the inference from gene trees to species trees, like other areas of phylogenetic analysis, is very controversial.

The situation in polyploids may be further complicated by the presence of both ***homologous*** and ***homeologous*** chromosomes and genes. In autopolyploids, derived from a single diploid species, individual plants have multiple sets of chromosomes that are all similar (Fig. 1.a). Allopolyploids, on the other hand, having originated from hybridization among different species, have chromosomes, and thus genes, that are derived from each of their diploid progenitors (Fig. 1.b). The members of a pair of chromosomes from one diploid progenitor are ***homologous***, and are usually more similar to each other than the corresponding chromosomes derived from the other progenitors, which are ***homeologous***. Of course, autopolyploidy and allopolyploidy are really the extremes of a continuum in the degree of differentiation of the chromosomes (and genes) of the parental plants (Stebbins, 1947; Grant, 1971).

In the results of cladistic analysis the sequences of an autopolyploid will usually all appear together, at a single position on the phylogenetic tree, joining the position of the sequence of the progenitor species. The sequences of an allopolyploid are usually found at different locations on the tree, joining each of the progenitor species or populations. However, there are various processes which might cause exceptions to these expectations (e.g., polymorphism in the progenitor (in the case of an autopolyploid), concerted evolution across homeologous loci, or chromosomal rearrangements after the formation of an allopolyploid (see below)).

Molecular phylogenetic investigations of the relationships of *O. tuberosa* with wild Andean *Oxalis*

Oxalis species were collected during field work first in Bolivia and subsequently in Peru in order to study the relationships of ***Oxalis tuberosa*** to its wild relatives. The members of the $x = 8$ alliance and other putative relatives of oca are most abundant in the cloud forest regions of the "ceja de selva," but also at higher elevations and drier habitats. Although most published reports assert that oca is unknown outside of cultivation and that no wild *Oxalis* are known with tubers, wild tuber-bearing ***Oxalis*** taxa exist in both Bolivia and Peru. The tubers borne by some of the populations in Bolivia have very small tubers, whereas others are larger, although still smaller than those found in cultivated material. Although the ploidy level of these plants is unknown, it is suspected that they are not octoploid, but are probably an intermediate level of polyploidy (see below). If it is confirmed that they are not conspecific with cultivated oca, then they would belong to an as yet unnamed species. The tuber-bearing populations found in Peru in the Department of Cusco are a different species, ***O. picchensis*** Knuth, which has thin, above-ground stolons and small tubers (Photo 1A).

My work with DNA sequence data focused on two gene regions: (1) the internal transcribed spacer (ITS) of nuclear ribosomal DNA, and (2) the chloroplast-expressed (but nuclear encoded) isozyme of glutamine synthetase (ncpGS). Details of materials and methodology may be found in Emshwiller and Doyle (1998 and in press), and Emshwiller (1999).

Results and discussion—ITS data

The ITS region is currently one of the most frequently used gene regions in molecular systematic studies at lower taxonomic levels in plants. The ITS comprises two non-coding regions that are found between the genes that code for ribosomal RNA. These spacer regions are less constrained than the coding regions around them, so they have more variation that can be useful to study relationships among genera and species. The ribosomal genes themselves, on the other hand, are under more evolutionary constraint and so they are more conserved in sequence and can be useful at higher taxonomic levels. The ribosomal genes exist in arrays of hundreds or even thousands of copies per genome, which are usually all identical due to the processes of concerted evolution. This similarity is an advantage in that there is an abundance of identical copies in each cell, but concerted evolution can also contribute its own problems (see below).

The sample in the ITS study included wild *Oxalis* collected in Bolivia, *Oxalis* species that were purchased as ornamentals in the United States, and accessions of cultivated oca kindly provided by the germplasm bank of PROINPA (Programa de la Investigación de la Papa, Cochabamba, Bolivia). Sampling included members of the $x = 8$ group and morphologically similar species, as well as more divergent ***Oxalis*** species. Although my Peruvian collections were made after my work with ITS, the results with Bolivian accessions are relevant to the entire $x = 8$ alliance.

Some of the morphologically divergent ***Oxalis*** species were also very divergent in ITS sequence, so much so that their sequences could not be unambiguously aligned with those of oca and the $x = 8$ alliance. This molecular dissimilarity helps to confirm that these groups of ***Oxalis*** are not close relatives of oca, although it also precludes the possibility of using these sequences in the same analysis in order to test their relationships more rigorously. The morphology of some of these divergent groups is contrasted with that of the $x = 8$ "***Oxalis tuberosa*** alliance" below.

In contrast, the ITS sequences of species that were identified as members of the $x = 8$ group all joined the same clade on the ITS tree (Fig. 2), supporting the $x = 8$ alliance as a natural (i.e., monophyletic) group. The clade also includes other species of unknown chromosome number. Thus the ITS data, in the absence of cytological data but in agreement with morphological similarities, suggest that these other species are also members of the alliance. Within this clade there is very little variation in ITS sequence, so that the relationships among the member species cannot be fully resolved using these data alone. Some groups of clearly distinct species share the identical sequence, most notably a group that includes oca and several other species (see below).

The members of Knuth's sections are scattered in various parts of the ITS tree (Fig. 2). Thus the results of analyses of ITS data are more congruent with the cytological grouping found by de Azkue and Martínez (1990) than with the sectional classification of Knuth (e.g., 1930, 1931, 1935, 1936). For instance, ***O. ortgiesii***, the type species of the section ***Ortgieseae*** into which Knuth (1930) classified oca (as noted above), not only has a different base chromosome number, $x = 7$, but it also appears on the ITS tree as less closely related to oca than several species of different sections.

The primary ITS sequence of oca is found within the $x = 8$ group, and is identical to that found in the Bolivian wild tuber-bearing populations. However, the same sequence is also shared with two other wild ***Oxalis*** species in the sample (Fig. 2), so the ITS data can not be used to identify oca's progenitors precisely. A second sequence type was observed faintly in one oca accession, also within the $x = 8$ clade, suggesting that perhaps at least two progenitors of oca might both be found within the alliance. It is possible that concerted evolution, acting across homeologous loci (as has been demonstrated in polyploid cotton by Wendel et al. (1995), might have "erased" part of the evidence of oca's origins. This is one of several possible explanations for the weak appearance of this sequence type and its absence in two of the three plants of cultivated oca sampled for ITS.

The ITS data have not only helped to show which group of ***Oxalis*** species is most likely to include oca's progenitors, but these data have also helped to distinguish other species as being unlikely to include the genome donors of oca, even in the absence of cytological information for these species. A group of cloud forest species allied to ***O. andina*** Britton and ***O. dolichopoda*** Diels, for which cytological data are as yet unavailable, form a clade on the ITS tree that is sister (most closely related) to the clade with the known $x = 8$ species. Thus these species are suggested by the ITS results to be more closely related to oca and the $x = 8$ alliance than is ***O. ortgiesii.*** However, the ITS data indicate that members of this other group were not involved with the origins of oca regardless of whether or not these species also share $x = 8$ as base chromosome number. ***Oxalis pachyrrhiza,*** whose ITS sequence was the most divergent of those that could be included in the same analyses with those of the $x = 8$ group, was confirmed as not having been involved with the origins of oca.

The ITS data, in summary, have contributed to an understanding of the relationships of the $x = 8$ "***Oxalis tuberosa*** alliance" and oca's probable origins from within that group. The sharing of the identical sequence by cultivated oca and the Bolivian wild tuber-bearing taxon is congruent with the possibility that these wild populations might be among the progenitors of domesticated oca, but is not conclusive. Other sources of data are necessary because of the lack of sufficient variation among ITS sequences of members of the alliance to resolve their relationships, as well as because of the concerns mentioned above that some evidence may have been lost through concerted evolution. It is also advisable to test the congruence of results using data from several unlinked genes, to help confirm that the gene trees represent the species relationships.

Results and discussion—ncpGS data

Because of the need for additional data as described above, these studies were continued using a second nuclear gene: the chloroplast-expressed isozyme of glutamine synthetase (ncpGS), an enzyme that is important in nitrogen assimilation. This gene region, unlike the ITS, is a new tool for plant phylogenetic studies (Emshwiller and Doyle, in press). The part of the gene used in this study includes four introns. Like the ITS region, the introns of genes can be useful for studies at lower taxonomic levels, because they are under less evolutionary constraint than the coding regions around them and so they have more variability among species. The sampling for ncpGS included a subset of the species included in the ITS study, with fewer of the species outside of the $x = 8$ group and more species within the group, and

included the addition of wild Peruvian ***Oxalis*** accessions.

There is somewhat more variation among the ncpGS sequences of the sampled *Oxalis* accessions than among their ITS sequences (Emshwiller and Doyle, in press). More importantly, there was clearly more than one sequence type within each individual plant of cultivated oca. This was true of some other ***Oxalis*** species as well, including the wild tuber-bearing ***Oxalis*** of Bolivia. Although this meant that molecular cloning was necessary to separate the different sequences within a plant, this gene region has provided substantially more information about the origins of oca than was given by the ITS sequences.

The sequences were analyzed both with and without the cloned sequences of oca and the Bolivian wild tuber-bearing populations. The results of analyses of ncpGS sequences that excluded the cloned sequences are shown in Figure 3. The analyses of ncpGS data are generally congruent with those of ITS data, with only a single upper clade in conflict (Fig. 3 and Emshwiller and Doyle, in press). The ncpGS data provide more support for the $x = 8$ "***Oxalis tuberosa*** alliance" as a monophyletic group than was provided by ITS data. There is somewhat more resolution of relationships within the $x = 8$ alliance, although this is partly because of different sampling. The general congruence of these two gene trees helps to confirm that they probably reflect the relationships of the taxa overall, if not in every detail.

Techniques of molecular cloning of the products of PCR (the polymerase chain reaction) were used to determine the multiple sequences found in three individual morphotypes of cultivated oca and one plant of the Bolivian wild tuber-bearing populations. However, there are complications in the interpretation of the results, in part because of the possibility of errors caused by this technique. One kind of mistake is known as "*Taq* error," in which the polymerase enzyme may introduce the wrong nucleotide into the sequence. The second kind, which is more problematic, is "PCR recombination" (Jansen and Ledley, 1990; Bradley and Hillis, 1997), in which parts of the different sequences that truly exist in the plant may be recombined to form a sequence that does not occur in the plant. Although it is not possible to determine with certainty which of the cloned sequences are correct, there are some criteria by which we can judge which are more likely to be the true sequences (discussed in Emshwiller, 1999).

Among the cloned ncpGS sequences it is possible to distinguish classes of those that are similar (but not necessarily identical). There are four classes from cultivated oca, and two classes found in the wild tuber-bearing plant. Sequences that did not appear to be recombined were selected from of each these classes for inclusion in phylogenetic analyses of ncpGS data. Although multiple trees were found in these analyses, the four sequence classes of oca consistently separated into four different places on the gene trees (one of the trees is shown in Figure 4). Two of the sequence classes found in cultivated oca join the two sequence classes of the wild tuber-bearing ***Oxalis*** of Bolivia on the ncpGS gene tree. A third sequence class is the same as the sequence found in ***O. picchensis***, the wild tuber-bearing species from southern Peru. The remaining sequence class, which is identical with the sequence found in some accessions of ***O. spiralis*** and ***O. mollissima***, may have been a contaminant (Emshwiller, 1999).

The fact that the sequence classes found in oca are found in separate locations on the gene tree supports the idea that oca is an allopolyploid, formed by hybridization between multiple species. Because the two sequence classes of the Bolivian wild tuber-bearing taxon are likewise separated on the gene tree, these ***Oxalis*** are probably also of hybrid origin, and may also be allopolyploid, although the ploidy level of these plants has not yet been determined. Although the Peruvian *O. picchensis* has a single sequence class, estimates of the DNA content of this species by flow cytometry indicate that it is tetraploid (E. Emshwiller, unpublished data), so it is probably autopolyploid. The ncpGS data indicate that the two wild tuber-bearing taxa, *O. picchensis* and the undescribed Bolivian taxon, might both have been involved in the origins of oca. However, there are still some other alternative explanations and unanswered questions. For instance, it is possible that other as yet unsampled species may also share the same sequences. Also, because this evidence is derived from only one gene, possibilities such as introgression from wild species have not been completely eliminated. Nevertheless, these two wild tuber-bearing ***Oxalis*** taxa are currently the best candidates as the species from which the genomes of octoploid oca are derived.

In order to determine whether all of the different sequence classes were present in a larger sample of plants, additional cultivated oca accessions and wild tuber-bearing ***Oxalis*** were sampled through direct sequencing to avoid the time and expense of cloning sequences from large numbers of individuals. This increased sampling helped to confirm the allopolyploid nature of oca, because two of the sequence classes were always present in both cultivated oca and the Bolivian wild tuber-bearing taxon. However, further questions were raised about the contribution of ***O. picchensis***, because the sequence type that oca shares with this species was absent from one of the nine cultivated accessions sampled. There are various possibilities that might explain this result, but further study will be necessary to distinguish among them. Some of these possibilities include (1) octoploid oca had multiple origins, (2) the cultivated oca that lacks the ***O. picchensis***-like sequence is not octoploid, (3) wild-crop geneflow led to the introgression and dispersal of the ***O. picchensis***-like sequence into cultivated oca, (4) the ***O. picchensis***-like sequence was lost in some oca lineages after formation of the polyploid (loss of some parental sequences may occur rapidly after polyploidization, as has been demonstrated in ***Brassica*** and ***Triticum*** (Song et al., 1995; Feldman et al., 1997)).

Thus, the results of analyses of DNA sequences of ncpGS agree with those of ITS in their support of the $x = 8$ "***Oxalis tuberosa*** alliance" as a monophyletic group. However, the group appears to be considerably larger than the dozen species originally studied by de Azkue and Martínez (1990). The molecular data from both genes studied indicate that the group includes additional species that as yet lack published chromosome counts (Table 1), in agreement with morphological similarities (see below). The DNA sequence data of both ncpGS and ITS agree with the cytological data in their incongruence with the traditional sectional classification of Knuth (1930). These molecular data support the conclusion that the genome donors of octoploid ***O. tuberosa*** are to be found within the $x = 8$ group, and they have also helped to screen out other species from inclusion in the alliance. The ncpGS sequence data support the allopolyploidy of oca, and support two different Andean wild tuber-bearing taxa as possible progenitor candidates. Both the Peruvian ***O. picchensis*** and the Bolivian taxon may have been involved, through hybridization, in the origins of cultivated oca.

Morphology of the $x = 8$ alliance and other groups of *Oxalis* species frequently encountered in Peru

In this section the morphology of the $x = 8$ alliance is contrasted with other groups of ***Oxalis*** species that are commonly found in Peru. This brief summary is not intended as a thorough or definitive treatment, but simply as a rough guide for those who might be interested in distinguishing those ***Oxalis*** species that might be relatives of oca. The $x = 8$ alliance is discussed first, followed by the groups that are related closely enough to have been included in the molecular analyses described above, and finally some of the more divergent sections of *Oxalis* are described.

"*Oxalis tuberosa* alliance"

The members of the $x = 8$ alliance (Photos 1 A—F) are mostly succulent herbaceous perennials, although a few appear to be annuals and some are slightly woody (but still fleshy) sub-shrubs (Photo 1F). The stems may be erect or reclining; in some species they are scandent vines several meters long (Photos 1C & D). In most members of the alliance (as in some other ***Oxalis*** species) the petiole is articulated above the base, the part below this articulation remaining on the stem after abscission of the leaf. In many alliance species these petiole bases are relatively long, in some cases even spiny, and may conspicuously clothe the stem. The petiole bases are adnate to a pair of stipules, which may be wing-like and hyaline-membranous, the apex acute to truncate. In other species there is little wing, but the stipule apex extends to a narrowly long acuminate tip, or in a few other cases the stipules are nearly obsolete. The petioles of some species (e.g., ***O. ptychoclada*** Diels, ***O. herrerae*** Knuth) may become broadly swollen with water reserves, at least seasonally (Photo 1F). The fleshy, subsessile leaflets may be obcordate, obovate or sometimes rhombic, nearly always with a distal sinus, although this notch may be quite small in some species. The lateral leaflets of alliance species are somewhat oblique at the base. As in most other parts of ***Oxalis***, there are pulvini located at the base of each leaflet and at the basal articulation of the petiole. The inflorescence is a two-branched cyme, which in some species is condensed to nearly umbellate, or in others may have secondary branching. The corolla of alliance species is usually yellow with red (or brownish or purplish) veins (e.g., Photo 1B), although the corolla of at least one species may be entirely brilliant red (Photo 1C). The spatulate petals are somewhat crenate at the apex, and, as in other ***Oxalis***, they have contort aestivation and are coherent above the clawed base. The various alliance species differ in characters such as habit, form of the stipules and petiole bases, leaflet shape, angle and density of pubescence on the stem and leaves, kinds of trichomes on the styles and filaments, etc.

The members of the group are found throughout the northern and central Andes, from almost the limits of vegetation at high elevations (e.g., ***O. nubigena***, Photo 1E) down to the cloud forests of the "ceja de selva," where they are most abundant. One species from outside the Andean region is supported by the ITS data as a member of the group (Emshwiller and Doyle, 1998): ***Oxalis vulcanicola***, found in the highlands of Chiapas, El Salvador, Costa Rica, and Panama (Lourteig, 1981; Burger, 1991). Some other Peruvian ***Oxalis*** species, not

yet sampled for DNA sequences, are morphologically similar to alliance species, and are probably members of the group (e.g., ***O. marcapatensis*** Knuth (Photo 1F), ***O. mollis*** H.B.K., ***O. phaeotricha*** Diels, ***O. san-miguelii*** Knuth, and ***O. urubambensis*** Knuth). In addition, there are several distinct species that do not yet have published names that also appear to be members of the group.

Outgroup *Oxalis* species that were included in molecular trees

As noted above, a group of cloud-forest species allied to ***O. andina*** Britton (Photo 2A) forms a clade that is sister to the clade that includes the known $x = 8$ species on the ITS gene tree. Although the plants sampled for ITS were all collected in the "yungas" region of Bolivia, one of the species, ***O. dolichopoda*** Diels, was originally described from Peruvian material, and plants of a species resembling ***O. andina*** occur on the eastern Andean slopes in the department of Puno (personal observations). The group apparently includes at least 2-3 undescribed species. They resemble the alliance species in the general shapes of the leaflets and in having yellow corollas with red veins. However, the stems are thin and somewhat woody, and the leaves and stems are not succulent. Some species, such as ***O. andina*** and the similar (or perhaps conspecific) ***O. yungasensis*** Rusby, are caespitose and creeping, while others have more elongated erect stems, such as ***O. dolichopoda***. The stipules are also relatively large, but not as hyaline as in some alliance species. They grow on very steep rock cliffs in cloud forest areas of the eastern Andean slopes, between about 2,000 and 3,000 meters in elevation.

The latter group of species is supported by the ITS data as more closely related to oca and the $x = 8$ group than are two species that were grouped with oca in section ***Ortgieseae***: ***Oxalis ortgiesii*** Regel and ***O. boliviana*** Britton (see above and Fig. 2). These two species share the same general erect herbaceous habit and succulence of leaves and stems with oca and some of its relatives, so perhaps it is not surprising that Knuth (1930) classified them together. These two species, however, have very unusually shaped leaflets, whose diverging lobes have acute apices. The leaflets of ***O. boliviana*** are broadly ob-triangular, whereas those of ***O. ortgiesii*** are a unique fish-tail shape (Photo 2B). ***Oxalis boliviana*** is found in moist understory habitats below about 2,500 meters, and ***O. ortgiesii*** is found in still lower elevation forests. These two species form a "grade," rather than a clade, on the ITS tree (Fig. 2).

Although they were classified by Knuth (1930) in different sections, the two species ***O. megalorrhiza*** Jacquin and ***O. pachyrrhiza*** Weddell are supported by the ncpGS data as being very closely related, in agreement with their very similar morphology. In fact, the large number of differing determinations of specimens of these species in herbaria indicates that there are disagreements about the boundary between these species. ***Oxalis megalorrhiza*** is the type species of the section ***Carnosae*** (Reiche) Knuth (for an explanation of the nomenclatural problems and history of this name see Dandy and Young, 1959), whereas *O.* ***pachyrrhiza*** (Photo 2C) was classified by Knuth (1930) in section ***Acetosellae*** DC. These species both have a stiff thick caudex and tuberous roots. The leaflets are usually all similar (i.e., the lateral leaflets are not oblique at the base) and have very large papillose cells on the

lower surface, which may appear sparkling in living plants or lacunose in dried specimens. The sepals are clearly unequal: the outer three are broadly ovate to triangular, sometimes nearly auriculate at the base, whereas the inner two are narrowly linear. The petals are yellow, without red veins but sometimes suffused with pale pink on the outer surface. These species are usually found in dry habitats, often in unconsolidated rocky and sandy sediments on steep slopes.

Only a single specimen of ***Oxalis laxa*** Hooker & Arnott var. ***hispidissima*** Barnéoud was sampled for ncpGS data, and its sequence was the most divergent of those included in the analyses. This delicate caespitose annual species is found in dry areas of the western Andean slopes of Peru, and it has allies found in similar habitats further south in Chile and Argentina. Because I have not had the oportunity to observe additional plants in the field, nor has Lourteig's (1988) treatment in ***Flora Patagonica*** been available to me, I will not discuss it further.

Some more divergent groups of *Oxalis* found in Peru

The groups described above are allied closely enough to the $x = 8$ alliance that their sequences of the two gene regions used could be aligned with those of the $x = 8$ alliance and used together in the same phylogenetic analyses. Other Peruvian ***Oxalis*** species, however, are even more divergent, in both morphological and molecular characters. Most of the other species encountered in Peru belong to the sections ***Ionoxalis*** (Small) Knuth or ***Corniculatae*** DC, or the subgenus ***Thamnoxys*** (Endl.) Reiche emend. Lourt. (see Lourteig, 1994). The phylogenetic relationships among these groups have not been rigorously tested, but they are morphologically divergent groups and the few members that have been sampled for molecular characters differ more from the "***Oxalis tuberosa*** alliance" than the preceding groups.

Section ***Ionoxalis*** includes herbaceous acaulescent species with scaly bulbs. The members of the section may be found in diverse habitats from sea level to the high puna (Photo 2D). The corolla is usually not yellow, but rather ranges from white to pink or purple. The inflorescence may be umbelloid or reduced to a single flower. Unlike the $x = 8$ group, but like some other groups of ***Oxalis***, these species have distinctive calli (said to be calcium oxalate deposits) on the sepal apices, bulb scales, and sometimes leaflets, which may be orange in living material but turn dark on drying (Salter, 1944; Denton, 1973). There are a few bulbous species that have been introduced from southern Africa as ornamentals, which are not members of section ***Ionoxalis***. Unlike the native species, these may be caulescent and they have bulbs which are tunicate, rather than scaly. One of them, ***O. pes-caprae*** L., has escaped and become established in several areas of Peru (Macbride, 1943 and personal observations).

Section ***Corniculatae*** includes small herbaceous weedy species, some of which are cosmopolitan (such as the type species, ***O. corniculata*** L.), whereas others are strictly Andean species, such as ***O. calachacensis*** Knuth and ***O. bisfracta*** Turczaninow (Photo 2E). The latter species is not reported from Peru, but photographs taken by Mauro Vallenas R. (Ministry of Agriculture, Puno) indicate that it is probably present on the altiplano (personal observations). They are generally found in disturbed habitats, and some are quite common

or even troublesome. They may superficially resemble the smaller, more delicate members of the $x = 8$ group (Eiten, 1963), but they are not succulent, have thin, wiry stems and broadly rounded leaflets without oblique bases, and do not have the persisting long petiole bases found in the alliance. The corolla is yellow, but the nectar guides, when present, are found ***between*** the veins, not *on* the veins. The capsule in some members of ***Corniculatae*** is cylindrical and more elongated than in the alliance species, and the pedicel is reflexed in fruit, so that the capsule is raised and directed upward.

The members of subgenus ***Thamnoxys*** include small upright shrubs found mostly in hot tropical lowland habitats of the Americas. Some of the species found in Peru are ***O. barrelieri*** L., ***O. dombeii*** St. Hilaire, ***O. psoraleoides*** H.B.K. (Photo 2F), and ***O. spruceana*** Progel. They differ from the alliance species in their woody, non-succulent stems and their pinnate leaves (unlike other parts of ***Oxalis***, the members of this group have a developed rachis that separates the lateral leaflets from the terminal one). Their leaflets are usually not obcordate, but rather ovate, elliptic or rhombic. They do not have the characteristic stipules and articulation above the base of the petiole as found in the alliance.

Need for conservation

These last groups are clearly not closely related to oca, but are a sampling of the great diversity of forms of ***Oxalis*** found in South America. However, it is the members of the $x = 8$ "***Oxalis tuberosa*** alliance" that may have potential for future plant breeding efforts in oca. Some of the members of the alliance are highly restricted endemics, being known from a single location (personal observations and Pool in Brako and Zarucchi, 1993). Several do not yet have published species names. Some localities where herbarium specimens were collected 30 or 40 years ago are now completely deforested, and the species are no longer present. The remaining montane forests of Peru and other neotopical areas harbor wild relatives of many other crops besides oca (Debouck and Libreros Ferla, 1995). The loss of habitat from deforestation, especially in the "ceja de selva" but also in high Andean forests, may be destroying valuable genetic resources that should be preserved for future generations in the Andes.

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Tabla 1. Species of Oxalis (including Xanthoxalis) that are supported as members of the «Oxalis tuberosa alliance»by the results of phylogenetic analyses of the DNA sequences of the two genes used in this study.

Especies [a]	Sección [b]	2*n*=
O. tuberosa Molina (cultivada)	*Ortgieseae* Knuth	64[c]
O. sp. (silvestre con tubérculos de Bolivia)		
O. picchensis Knuth	*Ortgieseae* Knuth	
O. spiralis R & P ex G. Don	*Ortgieseae* Knuth	16[d,e,f] or 48[c,g]
O. mollissima (Rusby) Knuth	*Clematodes* Knuth	16[c]
O. medicaginea H.B.K.	*Clematodes* Knuth	16[c]
O. lotoides H.B.K.	*Clematodes* Knuth	48[c]
O. subintegra Knuth	*Clematodes* Knuth	16[c]
O. vulcanicola Knuth	*Ortgieseae* Knuth	
O. longissima (Kuntze) Schumann	*Ortgieseae* Knuth	
O. unduavensis (Rusby) Knuth	*Ortgieseae* Knuth	
O. sp. aff. ***melilotoides*** Zuccarini		
O. lucumayensis Knuth	*Clematodes* Knuth	
O. sp. aff. ***distincta*** Knuth		
O. nubigena Walpers	*Capillares* (Reiche) Knuth	48-50[h]
O. petrophila Knuth	*Corniculatae* DC	
X. flagellata Rusby [i]		
O. paucartambensis Knuth	*Carnosae* (Reiche) Knuth	
O. cuzcensis Knuth	*Laxae* (Reiche) Knuth	
O. herrerae Knuth	*Herrerea* Knuth	16[c]
O. tabaconasensis Knuth	*Clematodes* Knuth	16[c]
O. weberbaueri Diels	*Carnosae* (Reiche) Knuth	
O. ptychoclada Diels	*Carnosae* (Reiche) Knuth	16[a,f]
O. oblongiformis Knuth	*Clematodes* Knuth	16[c]
O. peduncularis H.B.K. var. ***peduncularis***	*Carnosae* (Reiche) Knuth	16[c]
O. peduncularis H.B.K. var. ***pilosa*** Hieronymus	*Carnosae* (Reiche) Knuth	
O. teneriensis Knuth	*Carnosae* (Reiche) Knuth	
O. staffordiana Knuth	*Carnosae* (Reiche) Knuth	
O. polyrhiza Knuth	*Carnosae* (Reiche) Knuth	

[a] Additional species that are not identified or that are new species without published descriptions are not included in this list. [b] Sections of Knuth (1930, 1935, 1936). [c] de Azkue and Martínez, 1990. [d] Brücher, 1969 (as *O. pubescens* H. B. K.). [e] Favarger and Huynh 1965. [f] Huynh, 1965. [g] Mathew, 1958 (as *O. pubescens*). [h] Diers, 1961. [i] Combination as a species of *Oxalis* proposed by both Lourteig and Eiten on specimen annotations, but unpublished.

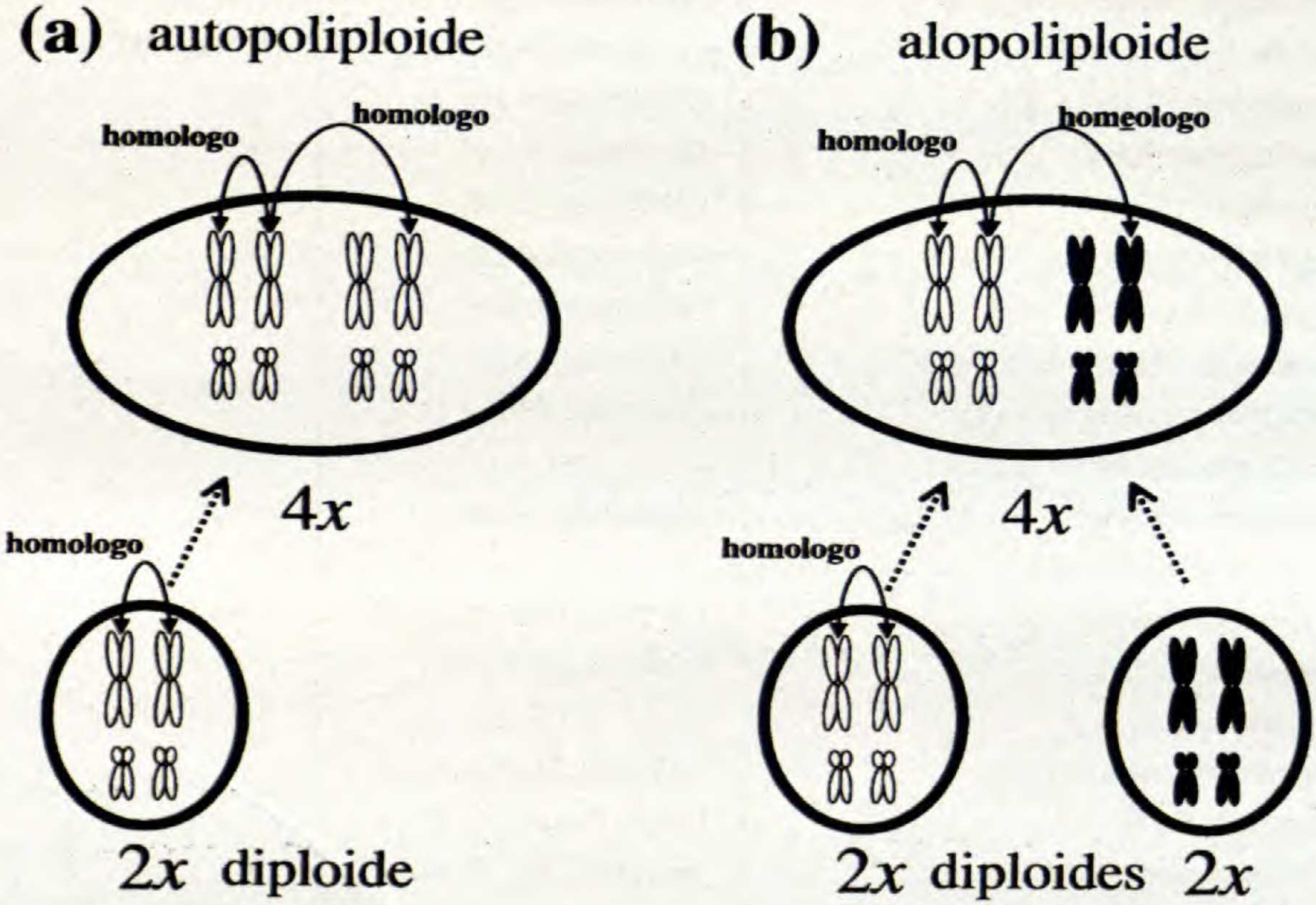


Figure 1. A comparison of homologous and homeologous chromosomes as found in polyploid plants. A simplified example is shown with diploids and tetraploids based on x = 2. The members of each pair of chromosomes in the diploids are homologous with each other (as are the genes at any particular locus on those chromosomes). **(a)** In an autotetraploid, all four sets of chromosomes are derived from the same species (perhaps even the same plant), so there are four homologues of each kind of chromosome, which usually pair randomly with each other. **(b)** In allotetraploids, however, the sets of chromosomes are derived from separate species (or well-diverged populations), so that the members of a pair derived from the same parental species (homologous) are more similar to each other than are the corresponding chromosomes derived from the other parental species (homeologous). The chromosomes of most allopolyploids will usually pair only with the homologous, not the homeologous, chromosomes.

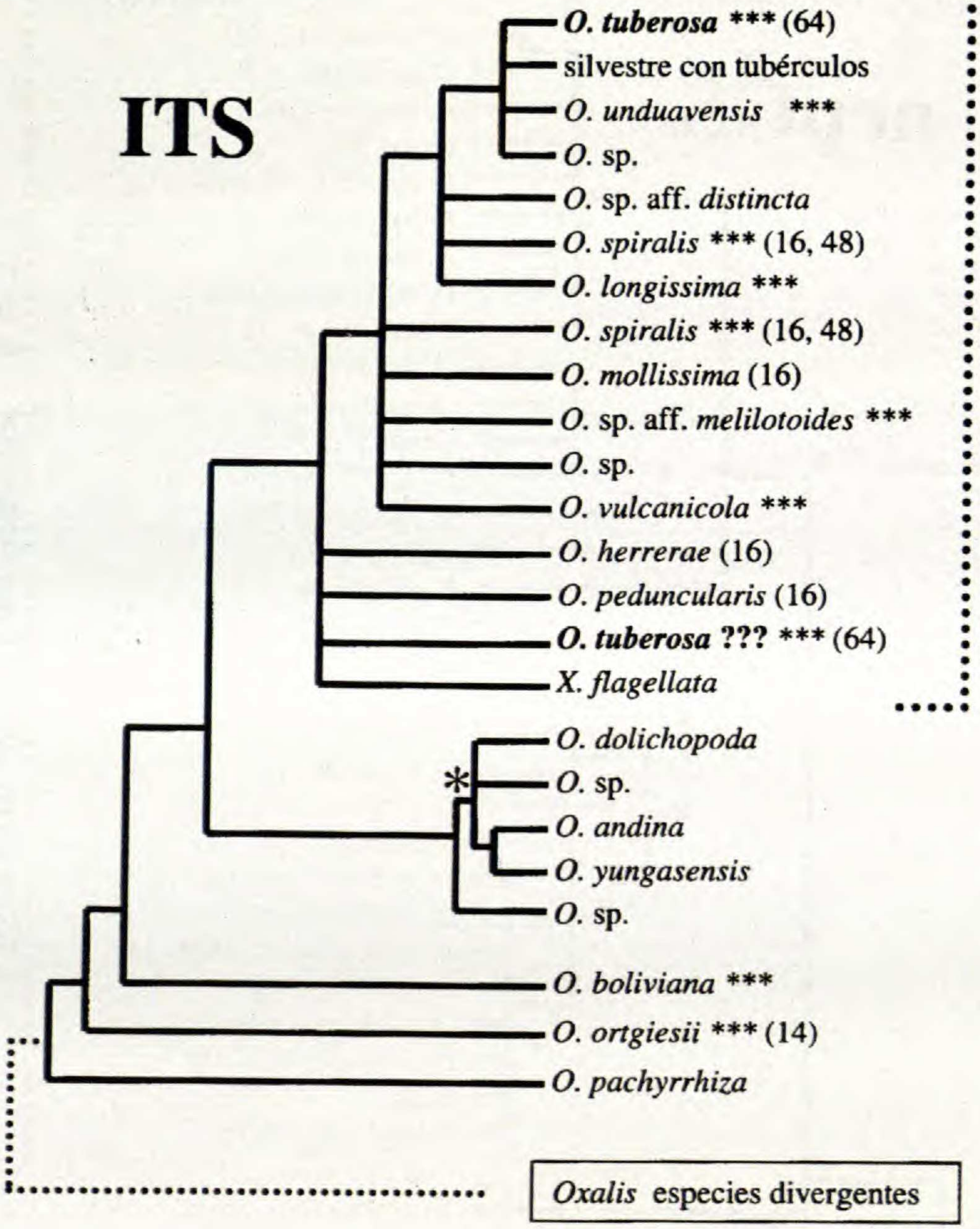


Figure 2. One of two maximally parsimonious trees found in analyses of ITS data of species of ***Oxalis*** (including ***Xanthoxalis***, which is here considered to be part of ***Oxalis***) modified from Emshwiller and Doyle, 1998. The alternative tree differs only in the loss of resolution at the node indicated by a single large asterisk. The primary sequence found in ***Oxalis tuberosa*** is found at the top of the diagram, whereas the faint secondary sequence of oca is followed by "???". Published chromosome numbers follow each species name (see text for references). Species that were classified by Knuth as members of section *Ortgieseae* are indicated by "***", and are interspersed with species of other sections. See Table 1 for the sectional classification of Knuth and references for these chromosome counts.

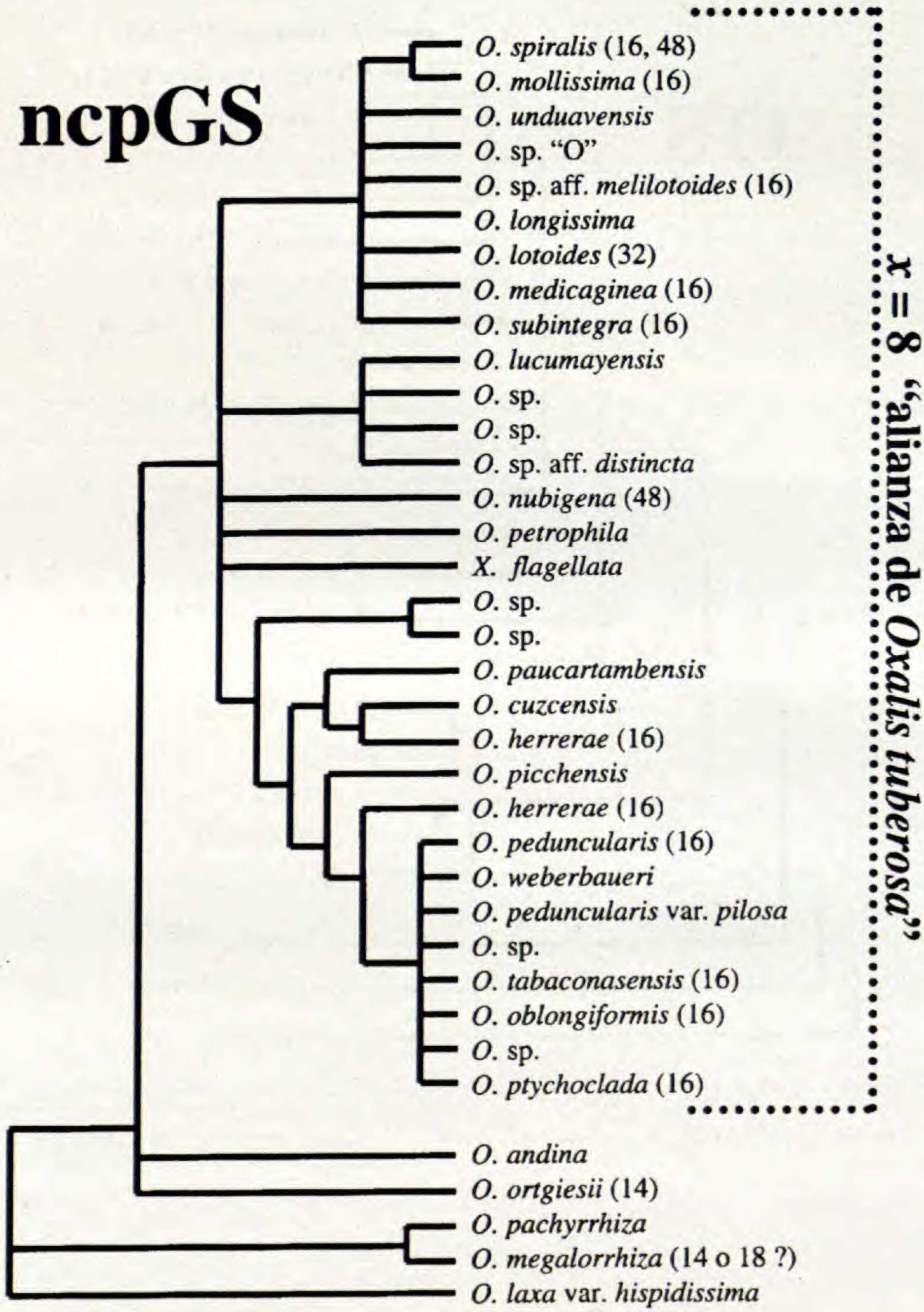


Figure 3. Strict consensus of 12 maximally parsimonious trees found in analyses of ncpGS data of ***Oxalis*** (including ***Xanthoxalis***) that *excluded* cloned sequences from cultivated oca and the Bolivian wild tuber-bearing ***Oxalis***. As with the ITS data, all of the species reported to have $x = 8$ were found in a single clade on this tree (reported chromosome numbers follow each species name. An arrow indicates the only clade that conflicts with the ITS results presented in Figure 2.

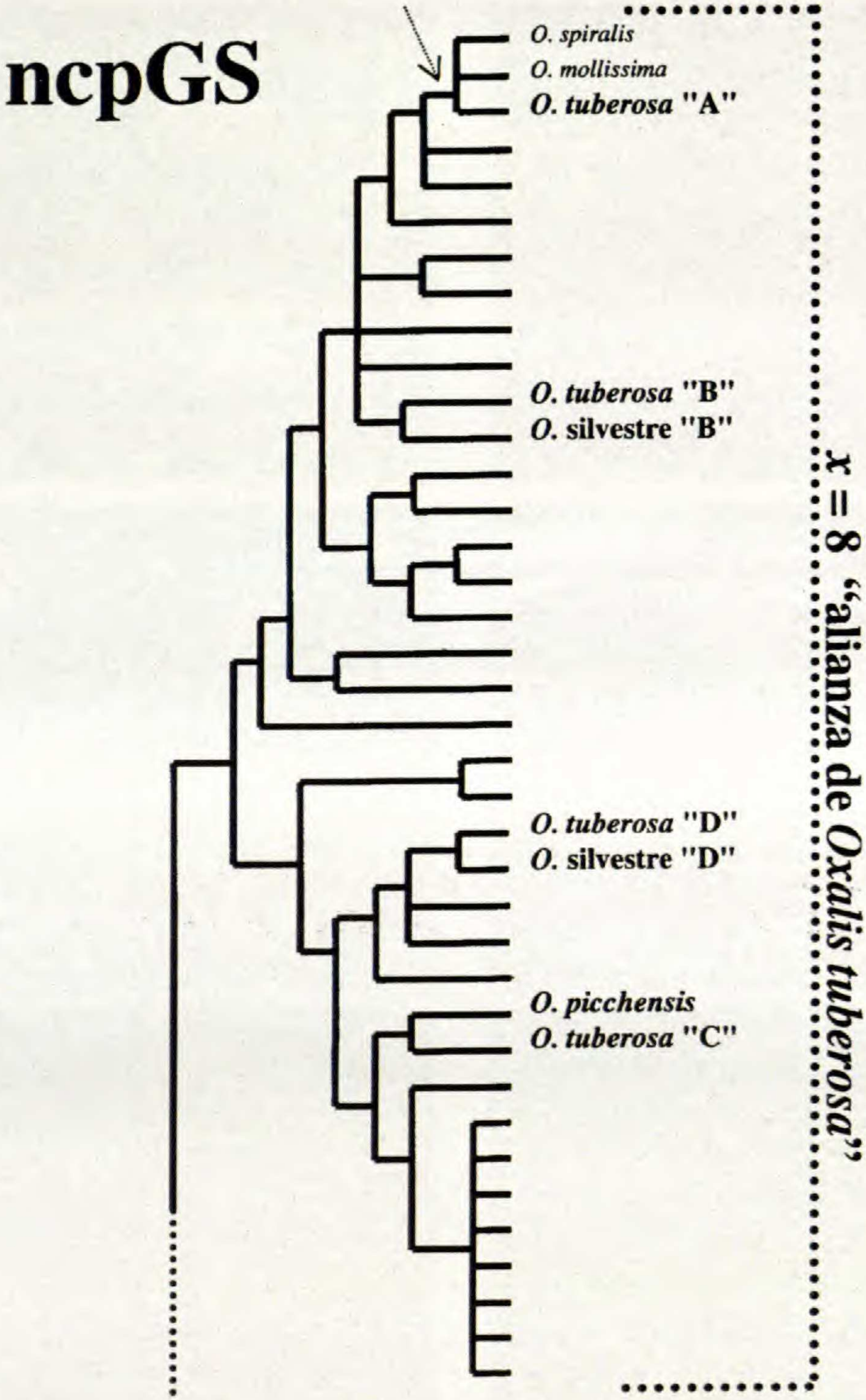
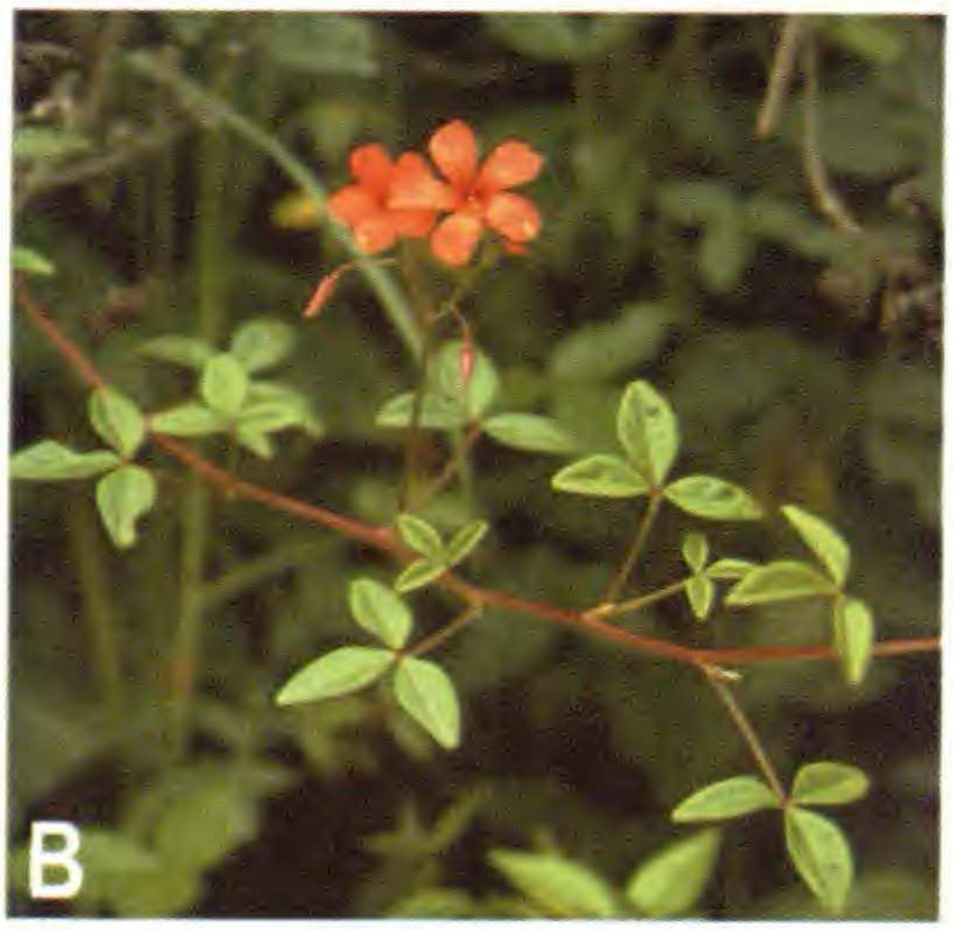
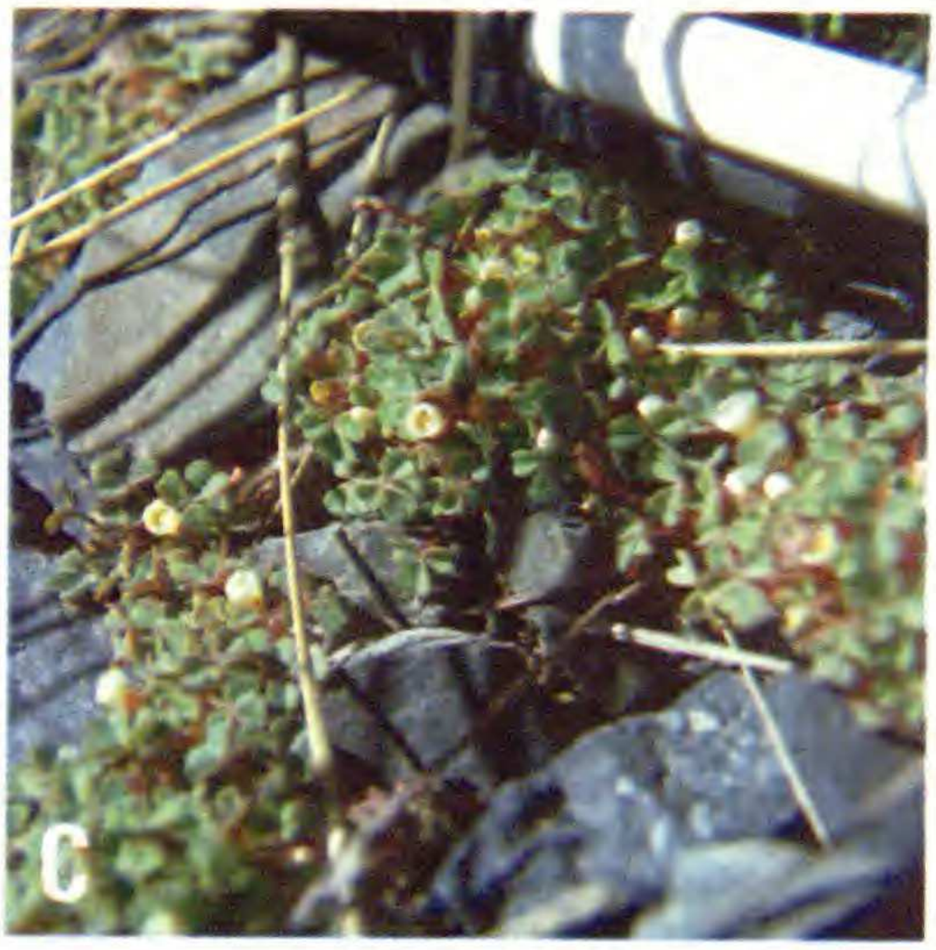
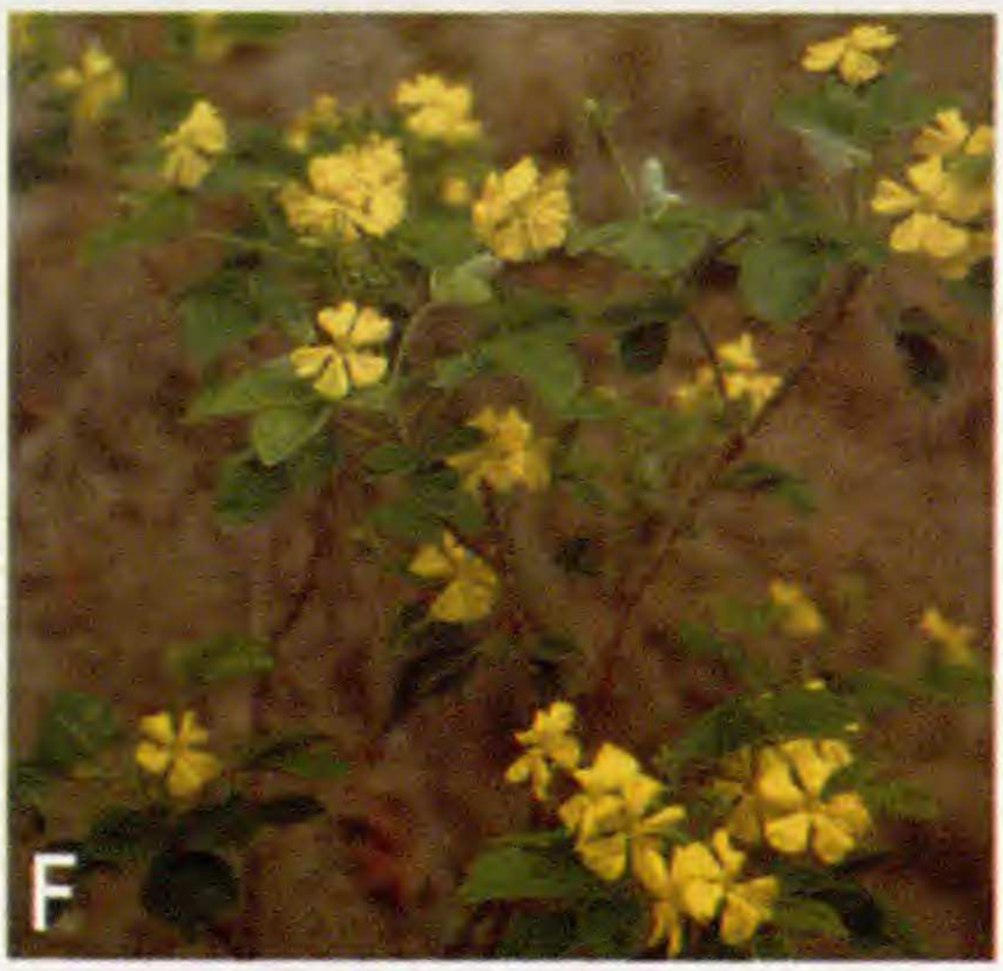


Figure 4. One of the 208 maximally parsimonious trees found in analyses of ncpGS sequences of ***Oxalis*** (including ***Xanthoxalis***) that *included* selected cloned sequences from cultivated oca and the Bolivian wild tuber-bearing ***Oxalis***. For simplicity, the diagram only shows the clade that includes the plants identified as members of the $x = 8$ alliance. The four sequence classes found within an individual plant of cultivated oca ("A" through "D") join different parts of the $x = 8$ clade in this and in all of the other maximally parsimonious trees found. Two of the classes ("B" and "D") are grouped with the two corresponding classes found in the wild tuber-bearing ***Oxalis*** populations of Bolivia. The third class ("C") joins the sequence of *O. picchensis* of southern Peru. The fourth class ("A"), which joins the sequences of ***O. spiralis*** and ***O. mollissima***, may be a contaminant.





Photos 1. Putative members of the "***Oxalis tuberosa*** alliance": (A) Small tubers of ***O. picchensis*** of Department of Cusco. (B) ***Oxalis petrophila***, an attractive member of the alliance with typical yellow corolla with red veins. (C) The entirely red color of the corolla of ***O. subintegra*** is unusual among members of the alliance. (D) ***Oxalis lucumayensis***, a large succulent vine of the "ceja de selva." (E) Growing at 4500m, ***O. nubigena*** may flower when only 2 cm tall. (F) Plasticity of swollen petioles in ***O. marcapatensis***, a species with the morphological features of the alliance, but not as yet included in molecular studies.



Photos 2. ***Oxalis*** species of groups other than the "***Oxalis tuberosa*** alliance": (A) ***O. andina*** and its allies are closest relatives of the alliance. (B) ***O. ortgiesii***, type species of the section *Ortgieseae*. (C) Tuberous roots of ***O. pachyrrhiza***. (D) Bulbous ***O. minima***, of section ***Ionoxalis***, in the high Andean "puna". (E) Weedy ***O. Bisfracta*** of section *Corniculatae*. (F) Shrubby ***O. epsoraleoides*** of subgenus ***Thamnoxys.***